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FINAL TECHNICAL REPORT

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# GRAVITROPIC RESPONSE MECHANISM IN CEREAL GRASS SHOOTS

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## DESCRIPTION OF RESEARCH

The primary goal of this research is to unravel the gravitropic response mechanism in cereal grass shoots. To achieve this goal, we must first decipher the nature of the gravisensors and how they act to perceive a gravitational signal. Then, we must determine how gravity perception leads to transduction of the signal; that is, when and how hormone asymmetry is established for both of the candidate hormones, native IAA (indole-3-acetic acid) and gibberellins. Finally, it is essential to elucidate how the hormone asymmetry, once achieved, leads to asymmetric growth in an upward-bending pulvinus. Primary components of this unequal growth response mechanism include: differential protein synthesis, sucrose and starch catabolism, beta-D-glucan turnover in the cell walls, and cell wall loosening and synthesis. Our current NASA research focusses on each of these components of the gravity response. A summary of the past three years' accomplishments and the significance of these results are present below.

## ACCOMPLISHMENTS

### 1. Graviperception in Cereal Grass Pulvini

We have demonstrated that starch-filled chloroplasts in statenchyma cells of the pulvinus (Figure 1-3) act as the primary gravisensors in the grass pulvinus system. Dark-induced loss of starch in the chloroplasts results in loss of gravitropic response and reconstitution of starch in these organelles (Figure 4) by sucrose feeding results in restoration of the graviresponse in

*/ Other participants in our research include Casey Lu, Najati Ghosheh, Nick Carpita, and David Gibeaut.

isolated pulvini (Table 1). Such pulvini do not loose their ability to respond to hormones such as IAA and gibberellins.

2. Transduction in Cereal Grass Pulvini

(a) Pulvini with the node excised show no significant graviresponse over 24 hrs. Pulvini excised with the node attached do gravirespond.

(b) In the vertical orientation, pulvini rapidly take up $^{3}$H-IAA, with or without the node. Polar auxin transport (export of label into receiver blocks) is similar in pulvini with or without the node.

(c) After gravistimulation, export of label into receiver blocks decreases, with or without the node. The ratio of label in the lower pulvinus half versus that in the upper half increases from 1.0 to 1.5, but only in pulvini with nodes. This ratio first significantly differs from 1.0 at 60 min. (using 10 min. increments) and is maintained at approximately 1.5 for at least 8 h. Also, movement of label into agar blocks placed atop or below the horizontal pulvinus is asymmetric in gravistimulated pulvini with nodes. This asymmetry is comparable in size and direction to that measured in the pulvini themselves.

3. Gravity Response Mechanism in the Pulvinus System

(a) Invertase, which catalyzes the hydrolysis of sucrose into D-glucose and D-fructose, changes markedly in response to gravistimulation. In lower halves, by 24 hrs., its level of activity is 28-fold higher than in pulvini of upright control pulvini; in upper halves, the level of invertase activity only rises 7-fold within the same period. The first changes in top/bottom asymmetry in invertase activity in gravistimulated pulvini are seen within 6 hrs. after beginning of gravistimulation treatment.

(b) Analysis of cell wall components of the pulvinus indicates that gravistimulation causes no changes in relative amounts of pectins, cellulose, arabinoxylan, xyloglucan, and wall protein. But, it does cause a dramatic

change in one of the hemicellulose polymers: beta-D-glucan (made up of mixed beta-1,3-glucan and beta-1,4-glucan). After 24 hrs. of gravistimulation, we see a 2-fold increase in beta-D-glucan in the lower halves over that in the upper halves. This is the first report of a significant change in a cell wall polysaccharide component elicited by gravity in a monocot shoot system like the cereal grass pulvinus.

SIGNIFICANCE OF THE ACCOMPLISHMENTS

1. In the absence of any evidence to the contrary, we have now unequivocally established the fact that starch-filled chloroplast statoliths act as the gravisensors in gravistimulated cereal grass pulvini. How they act as gravisensors that leads to transduction of the response is an open question. They could act as pressure probes to open hormone or ion channels in the plasma membrane. Or, they may possibly play a role in facilitating the establishment of auxin and gibberellin gradients by eliciting enhanced synthesis of the hormones and/or release of the hormones from their stored conjugates.

2a. We now have a system for separating graviperception from graviresponse in oat pulvini. Pulvini contain graviperceiving statoliths and are the site of response. However, isolation of the pulvinus from other tissues (e.g., the node) eliminates the graviresponse. Inclusion of the node with the pulvinus allows the graviresponse to proceed.

2b. Upon gravistimulation, basipetal transport declines, with or without the node. This indicates that polar auxin transport is linked to pulvinus orientation or graviperception.

2c. Lateral transport of label in pulvini preloaded with $^{3}H$-IAA occurs only in tissues that show a graviresponse. The timing of redistribution is consistent with this interpretation. However, the resulting asymmetry is less than that measured in other tissues and is less than the asymmetry in endogenous

levels of free IAA measured in gravistimulated oat pulvini. Apparently, lateral transport of IAA plays a role in gravitropism in oat pulvini but cannot entirely account for the graviresponse.

3a. The primary significance of the elevated invertase activity seen in bottom and top halves of graviresponding pulvini is that the pool of hexose available for cell wall synthesis is vastly increased. There is a very close parallel between the asymmetry seen in cell elongation and invertase activity from top to bottom of a graviresponding pulvinus. Since the asymmetry in invertase begins to appear within 2 h. after initiation of gravistimulation, we believe that the cell wall synthesis step in the gravitropic response mechanism starts relatively early- within 60 minutes after cell wall loosening is initiated (this wall loosening occurs close to 60 minutes after initiation of gravistimulation based on wall extension analyses).

3b. Beta-D-glucan is now considered to be a prime candidate for the site of action of hormones such as IAA and gibberellins to effect differential cell wall loosening and synthesis in the grass pulvinus system. It is this polysaccharide component of the hemicellulose matrix in the cell wall that changes significantly in response to gravistimulation. We can thus visualize that the hormones regulate turnover of this polysaccharide (by affecting rates of synthesis and degradation of beta-D-glucan) in such a way as to cause wall loosening which in turn opens up sites in the polymer for new synthesis. The differential thick-thin regions of the walls of pulvinus collenchyma cells seen after gravitropic curvature has occurred provides a structural basis for this contention (Figure 5). Wall loosening in these cells would occur in the thin regions; such regions could also be sites where new cell wall synthesis occurs.

The working model depicted in Figure 6 provides a conceptual framework for what we believe is happening in the graviresponding cereal grass pulvinus from the time of gravity perception to the last stages of upward bending.

## IV. PUBLICATIONS

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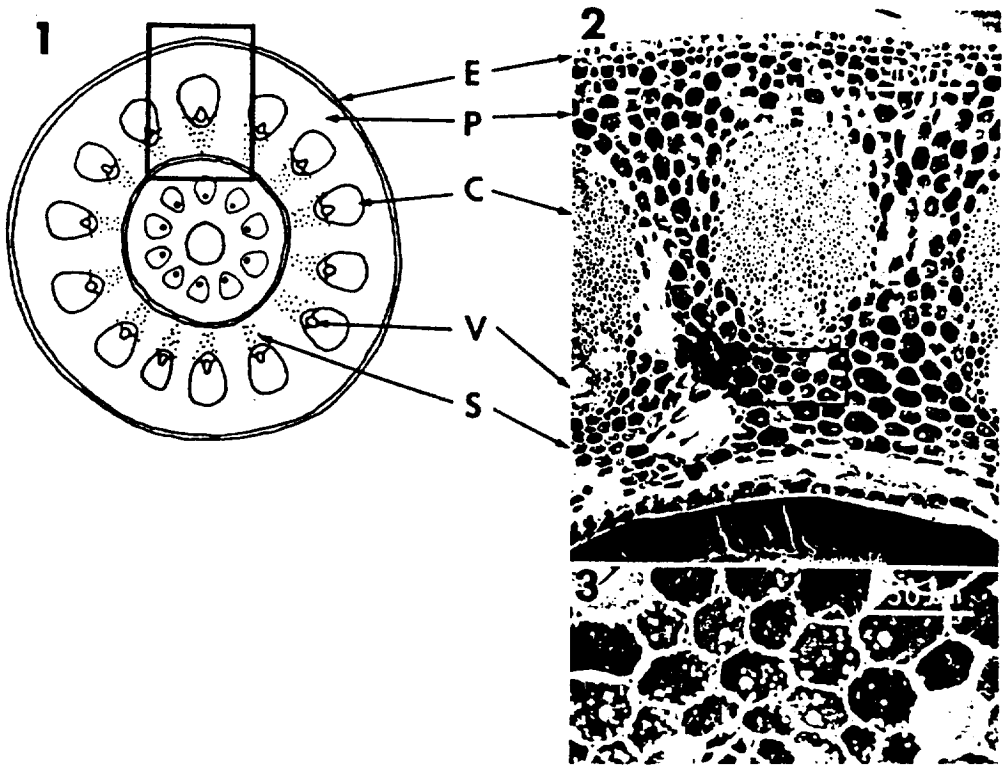



Figs. 1-3

Illustrations showing the location of statenchyma in the leaf-sheath pulvinus of a barley (*H. vulgare* cv 'Larker') shoot. 1, Cross-sectional diagram depicts the midportion of a barley leaf-sheath pulvinus and an internode base within it. The stippled regions denote the location of regions of statenchyma (S) in the pulvinus. Such regions are located to the inside of each vascular bundle. 2, Scanning electron micrograph shows a single vascular bundle with its associated statenchyma. 3, An enlarged view of this statenchyma tissue. Note that the statenchyma cells contain prominent starch statoliths which appear as white spheres. E, outer epidermis P, parenchyma tissue; C, collenchyma tissue; V, vascular bundle; S, statenchyma (from 20).



Table 1

Table I *Effect of Dark Pretreatments on the Gravitropic Response in Leaf-Sheath Pulvini of 'Larker' Barley Plants*

| Pretreatment before Gravistimulation | Gravistimulation[a] in the Dark at 25°C | Gravitropic Response[b] 24 h | 48 h |
|---|---|---|---|
| Dark[c] | Stem segments fed 0.1 M sucrose | 8° ± 0.69 | 39° ± 1.97 |
| | Stem segments fed distilled water | 0° | 0° |
| Light[d] | Stem segments fed 0.1 M sucrose | 32° ± 1.82 | 67° ± 3.99 |
| | Stem segments fed distilled water | 16° ± 1.59 | 25° ± 2.15 |

[a] For gravistimulation treatments, we used excised stem segments from barley shoots. Each contained a single pulvinus. See "Materials and Methods" for details on stem segment preparation and conditions employed for gravistimulation of these segments. [b] SE are indicated after each set of curvature response data. Twenty pulvinus-containing stem segments were used for each treatment in each experiment. If starch fails to be reformed for any reason (probably plastid membrane system damage during the long dark pretreatment), then no upward bending response occurs. [c] For dark treatments, flats of intact 45-d-old barley plants were kept in the dark for 5 d at 25°C. [d] For control, comparable-aged plants were maintained in the greenhouse under natural d length conditions of March to May, 1986 and 1987 at 25°C.

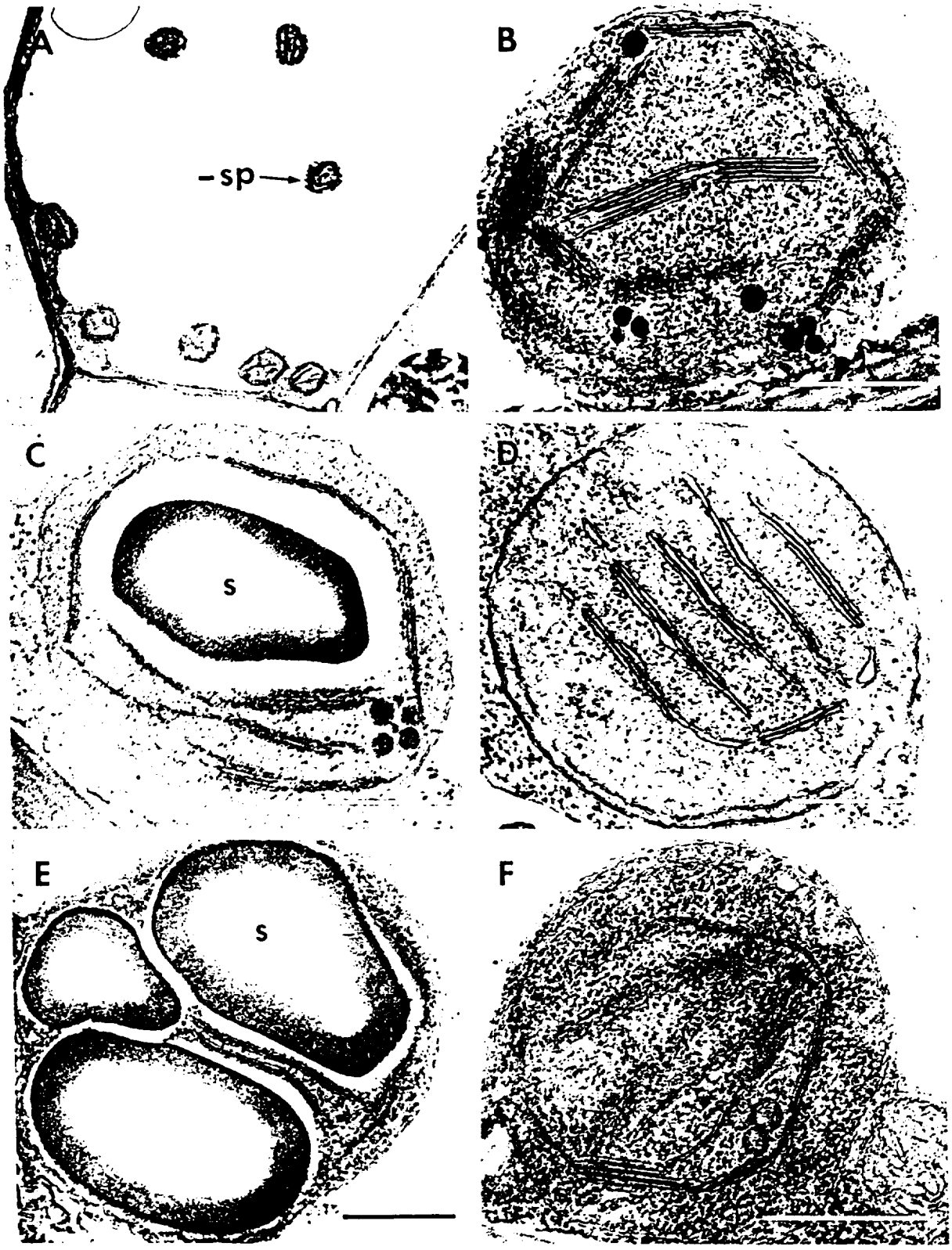



Fig. 4

Transmission electron micrographs of starch-depleted plastids in statenchyma cells of the p-1 leaf-sheath pulvini of dark pretreated 'Larker' barley plants. A and B illustrate the effect of 5 d of dark treatment on the occurrence of starch in the plastids. Note that the starch is absent. After the 5 d of dark pretreatment, excised segments from these plants were gravistimulated while being incubated in either 0.1 M sucrose (C and E) or in distilled water (D and F) in the dark at room temperature for 12 and 24 h, respectively. S, starch; -sp, starch-depleted plastid. Bars = 0.5 μm.

Fig. 5> Light micrograph using microscopy with crossed polarizers. Figure shows collenchymatous bundle cap cells (see location of these in Figure 1) of a control (top) and a 48 h gravistimulated oat pulvinus (bottom). X 135.

Fig. 6

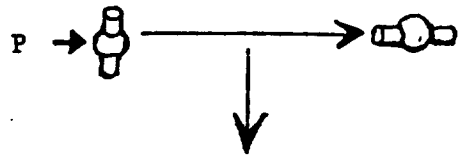
MODEL FOR GRAVITROPIC RESPONSE MECHANISM IN CEREAL GRASS SHOOTS

PERCEPTION

TRANSDUCTION

DIFFERENTIAL GROWTH

Initiation of gravistimulation treatment




P= leaf-sheath pulvinus

Starch statoliths cascade to bottoms of statocyte cells within 1-2 min.

Rapid establishment of top/bottom assymetry of free IAA and GAs, starting within 15 min

These hormones act to "turn on" genes responsible for synthesis of enzymes cited below

Enhanced activity of cell wall hydrolases such as beta-D-glucanase

Cell wall-loosening is initiated within 60 min and upward bending commences

Increase invertase activity results in release of hexoses (D-fructose & D-glucose) from sucrose for cell wall synthesis

New sites for cell wall synthesis are opened up and cell wall synthesis starts within 2 hrs.

Enhanced acivity of beta-D-glucan synthase

Differential cell wall-loosening and synthesis continue until upward bending ceases after 72 hrs.